Research Proposal Form of the Thesis Entitled

To compare efficacy of Mulethi and Guava leaf extract gels in treatment of Localized Aggressive Periodontitis.

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By

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INTRODUCTION:

Localized Aggressive Periodontitis (LAP) is an uncommon variant of inflammatory periodontal ailment distinguished by an expeditious pace of advancement, striking deterioration of attachment and bone structure, predominantly afflicting distinct teeth such as first molars and incisors, and manifesting at a youthful stage of life.[1,2] Previous research has elucidated that the prevalence of the disease is higher in economically disadvantaged countries (>5%) when contrasted with more affluent nations (≤3%).[3,4]Inflamed periodontal tissue is caused by gram-negative anaerobic bacteria, namely Porphyromonas gingivalis, Tannerella forsythensis, Prevotella intermedia, and Actinobacillus actinomycetemcomitans. An immune-pathological response is triggered inside the periodontal tissue by the entry of these bacteria and their toxins, which cause inflammatory cells such as lymphocytes and macrophages to become activated and release cytokines and other bioactive chemicals.[5] The leukotoxin produced by A. actinomycetemcomitans is a prominent endotoxin implicated in the development of periodontal disease.[6]When the periodontal tissues are exposed to these microbial toxins over an extended length of time, the alveolar bone and periodontal ligament are eventually destroyed, which leads to tooth loss.[7] In accordance with the World Health Organization (WHO), the prevalence of periodontal disease affects approximately 15%–35% of the adult population.[8] There is a pressing need for improved diagnostic methods and affordable treatment approaches for periodontal disease. Effective prevention of these diseases involves inhibiting bacterial biofilm formation on tooth surfaces, which can be achieved through mechanical removal and the utilization of antimicrobial agents.[9]Numerous studies have demonstrated the inhibitory properties of diverse antimicrobial agents against oral biofilm. However, these agents often come with undesirable side effects such as tooth discoloration, gastrointestinal issues, and the potential risk of antibiotic resistance development. Therefore, there is a pressing need for naturally derived herbal antimicrobials that can effectively impede dental plaque formation, offering a desirable solution to address this current requirement.[10] Ayurveda, an ancient Indian system of medicine, holds a vast repository of well-established and efficacious herbal remedies. In recent times, there has been a significant surge in awareness and scientific investigations concerning medicinal plants and their formulations. So for our research study we are evaluating and comparing efficacy of Glycyrrhiza glabra (Mulethi) extract gel and Psidium guajava (Guava) leaf extract in Localized Aggressive Periodontitis.

Glycyrrhiza glabra, a plant belonging to the Leguminosae family, is renowned for its medicinal properties. The root of G. glabra is the most frequently utilized part, known for its sweet taste and calming characteristics. Moreover, it exhibits antibacterial and anti-inflammatory properties.[11] Glycyrrhiza glabra root contains active phytoconstituents such as glycyrrhizin and glycyrrhetic acid, which are saponins responsible for its sweet taste. [12]These bioactive substances have the ability to prevent the proliferation of periodontopathogens and lower the amount of inflammatory markers at the infection site. Moreover, they promote the synthesis of osteoblasts, which aid in the development of new bone, while inhibiting the activity of osteoclasts, which contribute to the breakdown of alveolar bone in periodontitis.[13]Extended use of commercially available synthetic products is not recommended due to the potential for mucosal irritation and tooth staining.[14]

Guava extract is another natural extract that we want to use in our investigation. The plant known as guava, Psidium guajava, is acknowledged as a phytotherapeutic herb. It's also referred to as "poor man's apple" informally."[15]Scientific research has demonstrated the guava's hepatoprotective, antioxidant, antigenotoxic, antimutagenic, antiallergic, anticancer, and antihyperglycemic properties. Guava is also antibacterial, antiparasitic, antitussive, and hepatoprotective.[16] Moreover, the leaves of the guava plant have been traditionally utilized for maintaining oral hygiene.[17]

The antimicrobial effectiveness of guava extract against the cariogenic bacterium Lactobacillus acidophilus has been found to be comparable to that of chlorhexidine mouthrinse.[18]Despite the well-established antimicrobial properties of guava and Glycyrrhiza glabra (Mulethi) Extract Gel, there is a lack of comprehensive research on their specific effects on periodontal pathogens, including their virulence factors and enzymes. Only a limited number of studies have investigated the efficacy of Glycyrrhiza glabra (Mulethi) Extract Gel and guava as phytomedicines against periodontal pathogens. Moreover, there is a notable absence of literature comparing the effects of Glycyrrhiza glabra (Mulethi) Extract Gel and guava on oral Gram-negative microbes.

Hence, the current research aims to assess and contrast the effectiveness of a Gel made from Glycyrrhiza glabra (Mulethi) Extract with Guava extract in treating Localized Periodontitis.

In order for patients to afford a less expensive and more efficient means of managing their periodontitis, an increasing number of studies based on medicinal plants in the treatment or prevention of periodontal disorders should be carried out.

AIM

This study compares and assesses the effectiveness of Glycyrrhiza glabra (Mulethi) and Psidium guajava (Guava) leaf extract gel in the treatment of Localised Aggressive Periodontitis.

OBJECTIVES

1. This research aims primarily to evaluate and compare how well Glycyrrhiza glabra (Mulethi) and Psidium guajava (Guava) leaf Extract Gel manage the symptoms and development of localized periodontitis.
2. To assess the clinical efficacy of Glycyrrhiza glabra (Mulethi) extract gel in reducing pocket probing depth (PPD) in localized aggressive periodontitis patients.
3. To assess the clinical efficacy of Psidium guajava (Guava) leaf extract gel extract in reducing PPD in localized aggressive periodontitis patients.
4. To compare the effects of Glycyrrhiza glabra (Mulethi) and Psidium guajava (Guava) leaf extract gel on clinical attachment level (CAL) in localized aggressive periodontitis patients.
5. To determine the antimicrobial effects of Glycyrrhiza glabra (Mulethi) and Psidium guajava (Guava) leaf extract gel on periodontal pathogens associated with Localized Periodontitis.
6. To compare the antimicrobial effects of Glycyrrhiza glabra (Mulethi) and Psidium guajava (Guava) leaf extract gel on periodontal pathogens associated with localized periodontitis.

HYPOTHESIS

The comparative efficacy of Glycyrrhiza glabra (Mulethi) and Psidium guajava (Guava) leaf extract gel in the treatment of localized aggressive periodontitis will show significant difference in their ability to reduce inflammation, hinder the development of periodontal pathogens, and stimulate periodontal tissue regeneration.

NULL HYPOTHESIS

The comparative efficacy of Glycyrrhiza glabra (Mulethi) and Psidium guajava (Guava) leaf extract gel in the treatment of localized aggressive periodontitis will show no significant difference in their ability to reduce inflammation, inhibit the growth of periodontal pathogens, and promote periodontal tissue regeneration.

REVIEW OF LITERATURE

1. **H Manohar Sharma et al [19](2021),** performed a randomized controlled trial to assess the effectiveness of local drug delivery using 3% Psidiumguajava in treating chronic periodontitis.A total of 15 patients with 30 locations were included in this splitmouth trial.Participants were randomly allottedtotake either local medication delivery (3% P. guajava gel) combined with scaling and root planing (SRP) or SRP alone at corresponding sites. The principal aim was to assess the influence of A. actenomycetemcomitans (Aa) and P. gingivalis (Pg) on colony counts, clinical attachment level (CAL), and pocket probing depth (PPD). Assessing bleeding, gingival, and plaque scores were among the secondary goals. At the baseline, one month, and three months following the interventions, all of these measures were assessed. Throughout the investigation, significant advancements were noted in the clinical parameters. At the test locations, there was a statistically noteworthy rise in clinical attachment level (CAL) by 2.8 ± 0.152 and a statistically significant decrease in pocket probing depth (PPD) by an average of 2.74 ± 0.283 after three months. Furthermore, after one and three months ($P < 0.001$), microbiological investigation revealed a substantial decrease in the colony counts of A. actenomycetemcomitans (Aa) by an average of 17.4 ± 0.026 and P. gingivalis (Pg) by 22.7 ± 1.225 at the test sites. Clinical and microbiological improvements demonstrated the efficacy of locally administered 3% P. guajava gel as an adjuvant treatment for chronic periodontitis.

2. **Suman Madan et al (2019)**[20] conducted a randomized clinical trial Glycyrrhizaglabra -An efficient medicinal plant for control of Periodontitis. 104 patients were split into two groups for a randomised clinical control trial: an experimental group and a control group. For four weeks, 10% of the G. glabra gum paint concentration was administered to the experimental group. Every participant was told to strictly follow the guidelines for proper dental hygiene. Periadental pocket depth, loss of attachment, and

gingival haemorrhage were among the metrics used to assess G. glabra's efficacy. Statistics like mean, standard deviation, and t-test were used to analyse the preintervention and postintervention scores. The experimental group's patients' periodontal health significantly improved, according to the findings. The current study's findings showed that G. glabra was useful in preventing periodontal diseases.

3. **Y SunainaShetty et al (2018)**[21] conducted a study aiming to evaluate guava extracts' inhibitory impact on Porphyromonasgingivalis and Aggregatibacteractinomycetemcomitans. Additionally, they investigated the time-kill curve of P. gingivalis and A. actinomycetemcomitans, along with determining guava's antiproteolytic activity on P. gingivalis. The study utilized Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) methods to assess the inhibitory effects of aqueous guava extract (AGE) and ethanolic guava extract (EGE) on these periodontal pathogens. Furthermore,using the well diffusion procedure, antibacterial activity of guava extracts was evaluated. Moreover, a gelatin liquefaction test was used to evaluate the antiproteolyticoutcome of guava extracts on P. gingivalis's protease enzyme. The results showed that 75 µL/mL was the minimal inhibitory concentration (MIC) for P. gingivalis in both aqueous guava extract (AGE) and ethanolic guava extract (EGE). Conversely, EGE showed a minimum inhibitory concentration (MIC) of 75 µL/mL against P. gingivalis. For A. actinomycetemcomitans, the MIC for AGE was determined to be 50 µL/mL, while the MIC for EGE was found to be 3.12 µL/mL. EGE demonstrated higher susceptibility against P. gingivalis compared to AGE. Furthermore, guava extracts showed greater susceptibility against A. actinomycetemcomitans in comparison to P. gingivalis. According to the study's findings, guava extract exhibits strong activity against P. gingivalis and A. actinomycetemcomitans, suggesting that it might be a viable treatment for periodontitis.

4. In a study conducted by **Rakshanaa and Lakshmi (2017)** [22], the effectiveness of Ocimum sanctum and G. glabra mouthwash was tested and compared to chlorhexidine mouthwash. The results indicated that both the Ocimum sanctum and G. glabra mouthwashes were equally effective in combating oral pathogens.

5. Two randomized triple-blind placebo-controlled clinical trials were conducted by **Farhadetal.** [23] and **Moteshakkeret al**.[24]**2014)** on patients with periodontitis. In these studies, G. glabra capsules, at a dosage of 400 mg/day, were compared to doxycycline capsules.The study employed doxycycline capsules as the positive control and placebo

capsules as the negative control. The outcomes of the two trials showed that the patients' periodontal condition might be improved by taking both G. glabra and doxycycline capsules.

6. **Deepika Jain et al [25](2014)** carried out the testing of ethanolic and water extracts of guava leaves for their in-vitro anti-Lactobacillus acidophilus effect at different concentration. Using a Soxhlet extractor, guava leaf ethanol and water extracts were produced. For both extracts, two distinct concentrations of 5% and 20% weight/volume were made. Lactobacillus acidophilus, which was collected in lyophilized form from the Microbial Type Culture Collection 447, served as the test organism. For the experiment, the bacteria were resurrected in nutritional broth and then cultivated on Lactobacilli de Man, Rogosa, and Sharpe agar. The Agar well-diffusion method was utilized for the measurement of extracts' antibacterial activity. Each extract was made on ten plates. The positive control was 0.2% chlorhexidine, and the negative control was pure water.The mean zone of inhibition generated by the ethanolic extract at 5% and 20% was 11.2 mm, while the water extract at 5% and 20% produced 1.6 mm and 5.1 mm, respectively. One-way ANOVA and post-hoc Tukey's test were used to statistically analyse the data, and the results showed that the activity of 0.2% chlorhexidine was substantially higher than that of 5% ethanolic extract and 5%, 20% water extract. The 20% ethanolic guava extract and the 0.2% chlorhexidine showed no statistically significant differences in efficacy (P = 0.270). Outcomes of the study revealed that the guava leaf extracts, both ethanolic and water based, have antibacterial action against L. acidophilus, with 20% ethanolic extract being just as effective as 0.2% chlorhexidine.

7. **K. Ravi et al [26](2014)** demonstrated in their review on Psidiumguajava and its potential as an adjunct for the treatment of periodontal disease. Psidiumguajava, commonly known as guava, possesses a wide range of medicinal properties and has long been recognized for its anti-inflammatory, antimicrobial, antioxidant, and antidiarrheal properties. It also exhibits antimutagenic effects. However, despite its diverse biological benefits, there is a lack of comprehensive literature regarding its therapeutic potential in the treatment of periodontal disease. Therefore, this review aims to shed light on the potential of P. guajava in addressing periodontal disease and its associated conditions.

METHODOLOGY:

Place of Study: VYWS Dental College and Hospital Amravati.

Study Design: Interventional study.

Study Population: Patients between the age group 18–50 years.

Duration of Study: 6 months

Sample Size: Considering proportion from **Shetty YS, et al.**[28] We calculated the selected sample size for the current study with the help of Cohran's [Cochran, W.G (1977)][29] formula with 95% confidence interval, 5% margin of error and population proportion having 50% when the population is large. We analysed the sample size using Open Epi, Version 3, an open-source calculator, as 60.

A total of 60 samples will be selected for conducting the present study. These selected study participants will be categorized into two groups i.e., 30 samples in each group.

Sample size calculation formula by Cochran-

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

Sample Size for Frequency in a Population

Population size(for finite population correction factor or fpc) (*N*):	30
Hypothesized % frequency of outcome factor in the population (*p*):	50%+/-5
Confidence limits as % of 100(absolute +/- %)(*d*):	5%
Design effect (for cluster surveys-*DEFF*):	1

INCLUSION CRITERIA

1. Patients with mild to moderate aggressive localized aggressive periodontitis will be assessed.
2. The patients should be suffering from clinical attachment loss.
3. Patients will be included if they are in good systemic health and have not taken an antibiotic or any other medicine during the last six months.

EXCLUSION CRITERIA

1. Patients with systematic condition.
2. Patients with severe periodontitis.
3. Pregnant ladies and lactating mother.

OPERATIONAL DEFINITION

1. Localized Aggressive Periodontitis - A uncommon type of inflammatory periodontal disease called localised aggressive periodontitis (LAP) is characterised by an early onset age, a severe attachment and bone loss, and a quick rate of advancement on very specific teeth (first molars and incisors).

2. A. actinomycetemcomitans Leukotoxin - Leukotoxin (LtxA), a protein toxin secreted by A. actinomycetemcomitans, aids the bacteria in eluding the human immune system while infected. White blood cells (WBCs) are the specific target of the membrane-active toxin LtxA

MATERIAL and METHODS:

After obtaining appropriate permissions from the head of the department, Scientific Advisory Committee and Institutional Ethics committee, procedure will be as followed:

Study Instruments:

1. Glycyrrhiza glabra (Mulethi) Extract Gel.
2. Psidium guajava (Guava) leaf Extract Gel.
3. W.H.O. probe

Preparation of Guava Extract: The guava's young leaves need to be harvested and subsequently washed with tap water. To eliminate any impurities on the surface, the leaves will be treated with Tween 20. Afterward, they will be dried in a shaded area and then crushed into a coarse powder using an electric blender.

Aqueous Guava Extract: It is necessary to prepare a beaker with 100 gms of coarsely powdered guava leaf sample. One liter of distilled deionized water will be added to this. The mixture was then cooked until the volume was one-tenth (1:10) of its initial volume. It

was then filtered through Whatman filter paper with a 0.2-mm pore size to remove any remaining impurities. Clear crude extracts containing one millilitre (1 mL) must be placed in microcentrifuge tubes and dried with a speed vacuum concentrator (HETO) until a constant weight is obtained. After that, these dried extracts are to be kept in storage at a temperature of -20°C until needed again. All extracts must be carefully combined and diluted with sterile deionized distilled water before being used.

Aqueous Mulethi Extract: A botanist would verify the G. glabra roots that will be purchased from a nearby store. The roots will be dried at 37°C in a hot air oven to eliminate moisture. After the roots have dried, they should be crushed and macerated for a week with sporadic shaking in a solution of ethanol and water (30:70). The extract will be filtered through muslin cloth and Whatman filter paper number 1 after a week. A rotary evaporator would be used to dry the resultant crude extract. Serial dilutions of the crude extract ranging from 5 to 30 µl will be prepared and analyzed using an ultraviolet spectrophotometer to determine the concentration of the active phytoconstituentglycyrrhetic acid at its maximum absorption wavelength of 254 nm. The extract would be found to contain 28% glycyrrhetic acid concentration, which is parallel with the study conducted by Omar et al.[27] The creation of G. glabra gum paint will make use of pure vegetable food grade glycerin. Glycerin should be dissolved with a 10% concentration of G. glabra crude extract.

Bacteria and Growth Condition: The research will make use of a plaque samplewhich will be isolated using kanamycin at a concentration of 100 mg/L and contained 5% sheep blood as the primary ingredient in Trypticase blood agar base. Yeast extract, hemin, vitamin K1, L-cysteine, and yeast extract extract will be added as additional supplements to the pathogens.P. gingivalis and A. actinomycetemcomitans will be incubated for 48 to 72 hours at a temperature range of 35°C to 37°C in order to be subculturing. After being injected, the agar plates have to be kept in anaerobic jars and incubated for 48 hours. Following this, an additional incubation period of two to four days is recommended to allow slow-growing organisms to form colonies. Colonies of Porphyromonas gingivalis will be identified by their black and mucoid appearance, while colonies of A. actinomycetemcomitans will be characterized as small and translucent.

Procedure: There will be a randomized clinical trial to evaluate the effectiveness of gum paint. Based on the presence of mild to moderate periodontitis, 40 individuals will be chosen. The trial will involve patients who will be in good systemic health and who have not used an antibiotic or any other medicine within the previous six months. Gingival bleeding, periodontal pockets, and loss of attachment characteristics are to be employed in assessing periodontal condition. Patients will be randomly split into two groups at random: Group B will receive guava extract gel, while Group A will receive therapy with G. glabra gum paint. Patients must participate in follow-up for a full month. Patients in both groups will be given instructions to massage their gums twice a day (in the morning and evening) using G. glabra gum paint and guava extract gel. To prevent biases, Colgate toothpaste and brushes will be given to all patients (groups A and B).

On the baseline, 3 months and 6months of experimental phase, assessments will be made at each visit. Next, each patient will have their plaque index (PI), gingival index (GI), and papillary bleeding index (PBI) examined, and sterile probes will be used to collect plaque samples from a single surface.

Training and Calibration

All the examination and recording of the proforma will be carried out by a single examiner investigator.

Statistical Analysis

The chi-square test, student paired and unpaired t tests, and other descriptive and inferential statistics will be utilised in the statistical analysis. The software used for the study will be SPSS 24.0 and Graph Pad Prism 7.0, with a significance threshold of $p < 0.05$.E

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